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Biomass Yield, Juice Quality and Alcohol Production of Sweet Sorghum

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Abstract : Cultivation of energy crops under the normal conditions to produce biomass energy is thought to be one of the important ways to develop substitute energy. The present experiment was conducted to detect the biomass yielding capacity, juice physicochemical properties and the alcohol productivity of the selected sweet sorghum line AM-4 , and to evaluate its possible value as an energy crop.

The results showed that AM-4 had high biomass yield (44.4—46.1 t/ha), brix value (18.2—23.2) and sucrose content (11—12%), short growth period (four months) and wide adaptability. Results from the fermentation test indicated that alcohol production rate higher than 9% can be obtained through the juice fermentation with either *S. cerevisiae* or *Sf* yeast strain at 30°C within two days. The needed temperature can be easily achieved by the use of the abundant solar radiation in Taiwan. Therefore the input cost can be reduced considerably.

Introduction

Although the oil prices decreased, it doesn't mean that the energy crisis is over. The development of substitute energy resources such as biomass energy is still an important goal of research work in many countries [10].

Sweet sorghum, a promising biomass resource with high sugar content, has good adaptability, high yield and short growth period [1,7,8]. Its juice can be directly used for alcohol production [3]. This advantage for energy use has been recognized during the past years. The Department of Energy of the U. S. A. estimated that after 1990 sweet sorghum can top all crops such as cereals and sugar-cane as the best resource for alcohol production [2,3,11]. The national alcohol production program of Brazil has also selected sweet sorghum together with sugar-cane and cassava as the three main biomass resour-

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ces for ethanol production[4]. After having been introduced and tested in Taiwan, a few sweet sorghum varieties with short growth period, high yield and high sugar content have been selected[1,8]. The main purpose of this study was to determine the efficiency of alcohol production from the best sweet sorghum line, AM-4, and to find out the useful yeast strains and their appropriate reaction conditions.

Material and Methods

1. Material :

AM-4, a high yield sweet sorghum line introduced from the U. S. A. by Taichung District Agricultural Improvement Station, has been under cultivation in Taichung since 1981, in Wujih since 1983, and in Houli since 1983. The planting time was in fall 1981 and in spring 1983. The growing distance was 60×30 cm in Taichung area and 50×10 cm in Wujih and Houli area.

2. Methods :

The following characteristics are determined individually :

Density value : 100 ml of AM-4 juice was weighed and the density was expressed in g/ml. A total of 8 replications was determined and the mean value was presented.

Brix degree : The brix degree of AM-4 juice was determined with a Shimadzu refractometer.

Refraction rate : Take 2-3 drops of AM-4 juice and the refraction rate was determined with a Shimadzu refractometer.

Viscosity value : Take 100 ml AM-4 juice and determine the viscosity value from 16°C to 90°C with the Brookfield LVT viscosimeter.

Total nitrogen : Two ml of AM-4 juice were digested with concentrated H_2SO_4 with $CuSO_4$ and K_2SO_4 as catalysts. After digestion, the volume was made to 100 ml and 10 ml of the diluted solution was then distilled with 5 ml of 40% NaOH. Total N could be obtained by titrating with 0.01N HCl.

Sucrose content : Dilute the AM-4 juice 10 times and to 50 ml diluted juice add N/10 HCl, then heat it in the water bath. Neutralize the solution with N/10 NaOH and dilute to 250 ml. Take 1 ml of the diluted solution to determine the reducing sugar content with the Somogyi method and calculate the sucrose content with formula.

Reducing sugar content : Dilute the AM-4 juice 10 times and take 1 ml to determine the sugar content with the Somogyi method.

Moisture content : Heat the AM-4 juice at 105°C under normal pressure until constant weight is reached.

Determination of the amino acids : Take the dry AM-4 samples and put together with 10 times quantity of 6N HCl into the test tube. Close the gap under vacuum and heat it at 110±2°C for 24 hours. After drying add 0.05M sodium citrate buffer and

filtrate through a 0.2μ pore membrane. Then take 1-2 n mole sample to determine the amino acid composition with the amino acid analyzer.

Fermentation : Take 50 ml of AM-4 juice and add 1 gram of yeast (*Saccharomyces cerevisiae*). After adjusting the PH-value to 5.5 with H_3PO_4, put the solution into the incubator at 30°C. The other yeast strains tested are S290 , S24, S21 and Sf.

Ethanol determination : The ethanol rate was determined with a Shimadzu FID GC under the following conditions : Injection temperature 170°C, column 150°C, FID detector 200°C, N_2 speed 40 ml/min, air pressure 0.9 kg/cm^2, H_2 pressure 1 kg/cm^2, range 0.01v 256, sensitivity MΩ 10^2. Sample quantity in liquid form was 1 μ1. Before determining the ethanol rate the fermented solution should be centrifugated at 15,000 rpm for 20 minutes.

Result and Discussion

I.Stalk yield and the juice properties of sweet sorghum

Two cultural improvements, early planting in spring and narrow spacing, have increased the stalk yield of the AM-4. Highest yield of 44.4 t/ha is obtained in Wujih area and 46.1 t/ha in Houli area. The juice brix degree has also been increased from 12.5 to 18.2 degree (Wujih area) and to 16.1 degree (Houli area) (Table 1). These results have topped those of the U. S. A. and previous results obtained in Taiwan[8,11,12]. Am-4 has its highest brix degree up to 23.24 (Table 2). Therefore, AM-4 can be thought as a promising biomass producing source.

Table 1. Stalk yield and brix degree of sweet sorghum AM-4

Location	Planting Season	Spacing (cm)	Stalk yield (t/ha)	Brix degree
Taichung	fall, 1981	60×30	11.71	12.50
Taichung	spring, 1983	60×30	31.86	16.50
Wujih	spring, 1983	50×10	44.43	18.20
Houli	spring, 1983	50×10	46.08	16.10

The average growth period of sweet sorghum, including AM-4, is four months. It is 8-14 months shorter than that of cassava and 14 months shorter than that of sugarcane. The total energy inputs of sweet sorghum are 4,011 Mcal/ha, being the lowest of these three energy crops[4].

The juice of different sweet sorghum varieties possesses different physical and chemical characters. The physical properties of the AM-4 juice are analyzed and shown in Table 2. The brix degree ranges from 12.50 to 23.24 with an average value of 16.94. The density value is 1.06 g/ml, being higher than those of water (1.00), alcohol (0.79)

and gasoline (0.72-0.78). The average refraction index of AM-4 is 1.36. This is proportional to the brix degree and the density value (Table 2). At 16°C the average viscosity value is 6.55, which is almost two times of the value at 90°C. This means the viscosity value of AM-4 is inversely proportional to the temperature (Fig. 1).

Table 2. Physical properties of AM-4 sweet sorghum juice

Physical property	Mean	Max.	Min.	Difference
Brix (degree)	16.94	23.24	12.50	10.74
Density (g/ml)	1.06	1.08	1.03	0.03
Refraction	1.36	1.36	1.35	0.01
Viscosity (centipoise at 16°C)	6.55	7.20	5.50	1.70

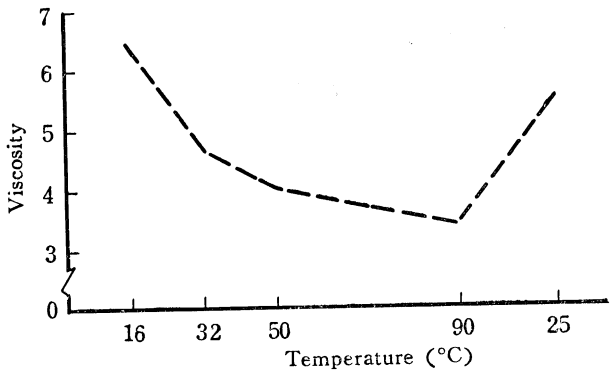

Fig. 1. Effect of heating temperature on the relative viscosity of sweet sorghum AM-4

The chemical analysis of AM-4 juice shows the following results. The highest component is the moisture with a content of 87.9% (86.1-89.3%). The sucrose content is 11.1% with a difference of 3.4%. The average reducing sugar content is 0.38% (0.31-0.56%). The average value of the total N is 0.045% (Table 3).

Table 3. Chemical composition (%) of sweet sorghum AM-4 juice

Chemical composition	Mean	Max.	Min.	Difference
H_2O	87.890	89.280	86.110	3.170
Sucrose	11.124	11.575	8.177	3.398
Reducing sugar	0.377	0.565	0.307	0.255
Total N	0.045	0.066	0.032	0.034

The amino acid composition of the AM-4 juice is also tested with an amino acid analyzer. Content of asparagine is highest (39.3%), followed by glutamine (14.4%) and alanine (8.6%). Lowest contents (about 0.37%) are found in cysteine and methionine.

Out of the 8 essential amino acids, 7 can be detected. Only tryptophan is absent. Values of the other amino acids are between those of alanine and methionine(Table 4). The amino acid content of sweet sorghum juice can be lower or higher, depending on the test methods and the different varieties [3].

Table 4. Amino acid composition of AM-4 Juice

Amino acid	Mean (%)	Max. (%)	Min. (%)	Difference
Asparagine	39.32	43.95	34.39	9.56
Glutamine	14.38	16.31	12.35	3.96
Alanine	8.55	10.48	7.26	3.22
Serine	5.85	6.82	5.25	1.57
* Valine	5.72	6.34	5.35	0.99
* Leucine	4.97	5.78	4.24	1.54
* Isoleucine	4.45	5.31	3.36	1.95
Glycine	4.38	5.11	3.48	1.63
* Threonine	4.09	4.61	3.71	0.90
Histidine	2.70	3.13	2.29	0.84
Arginine	1.75	2.06	1.50	0.56
* Phenylalanine	1.47	2.83	0.70	2.13
* Lysine	1.44	2.35	0.73	1.62
Tyrosine	1.12	1.68	0.58	1.10
Proline	0.90	...	...	...
Cysteine	0.38	...	...	...
* Methionine	0.37	...	...	...

* essential amino acid

II. The alcohol fermentation of sweet sorghum juice

Sweet sorghum juice is rich in sugar content and can be easily used for alcohol production. It is easier to use sweet sorghum than to use cassava, potato, sweet potato [9] or other starchy crops for producing alcohol. The ethanol rate, obtained from the AM-4 juice by using *Saccharomyces cerevisiae*, is affected by the pH value of the reaction medium and the reaction temperature. Under optimal pH value of 5.5, the ethanol rate reaches 10.27% and the ethanol concentration reaches 81.5 g/l. If the pH value is higher or lower the ethanol rate will be negatively affected. The optimal temperature is 30°C, where *S. cerevisiae* can produce the highest ethanol rate (10.75%) and ethanol concentration(85.4 g/l). The alcohol production is inversely proportional to the temperature. For example, if the temperature is over 55°C, the ethanol rate decreases rapidly. At a temperature of 30°C and a pH value of 5.5, yeast strain *Sf* can also produce a high ethanol rate (above 9%). The other yeast strains such as S21, S290, and S24 can produce an

ethanol rate around 7% (Table 5,6 and 7).

Table 5. The effect of pH values on the alcohol production of AM-4 juice[a]

Yeast strain	pH	Ethanol rate (%)	Ethanol conc. (g/l)
Saccharomyces cerevisiae	3.5	3.18	25.3
	4.5	7.81	62.0
	5.5	10.27	81.5
	6.5	7.96	63.2

a The reaction temperature was 35°C

Table 6. The effect of temperatures on the alcohol production of AM-4 juice[a]

Yeast strain	Temperature (°C)	Ethanol rate (%)	Ethanol conc. (g/l)
Saccharomyces cerevisiae	30	10.75	85.4
	35	9.43	74.9
	45	3.61	28.7
	55	0.91	7.2
	65	0.61	4.8

a pH of the reaction medium was 5.5.

Table 7. The effect of various yeast strains on the alcohol production of AM-4 juice[a]

Strain	Day	Ethanol rate (%)	Ethanol conc. (g/l)
Saccharomyces cerevisiae	1	9.06	71.9
	2	9.35	74.2
S 290	1	2.75	21.8
	2	7.04	55.9
S 24	1	2.56	20.3
	2	6.99	55.5
S 21	1	2.72	21.6
	2	7.57	60.1
Sf	1	3.04	24.1
	2	9.14	72.6
Control	1	0.01	0.1
	2	0.01	0.1

a Reaction temperature was 30°C and pH of the medium was 5.5.

The sugar concentration of the fermentation solution must not be too low, otherwise the yeast propagation will slow down and the alcohol production rate will decrease [2,3]. Th esugar concentration of AM-4 juice is between 10% and 12%, which is beneficial for yeast growth and alcohol production. With the fixed yeast quantity of 1g, the ethanol production rate of a 10% sugar concentration juice reaches 8.84%. With a sugar concentration below 6%, the ethanol rate decreases significantly. At 4% the ethanol rate is only 1/6 of that of 10% sugar concentration (Table 8).

The effect of various yeast quantities on the fermentation of AM-4 juice is shown in Table 9. With 1g of *S. cerevisiae* the ethanol production rate reaches 9.41%, which is the best result among the other yeast quantities tested. However, the yeast quantity does not significantly influence the ethanol production rate of AM-4 juice (Table 9).

Table 8. The effect of different sugar concentrations of AM-4 juice on the ethanol production rate[a]

Sugar conc. (%)	Ethanol production rate (%)
10	8.84
8	7.24
6	4.93
4	1.54
2	1.11

a Ucsing 1 g of *S. cerevisiae* for 50 ml of juice.

Table 9. The effect of various yeast quantities on the ethanol production rate of AM-4 juice[a]

S. cerevisiae (g)	Ethanol production rate (%)
1	9.41
2	8.88
3	9.27
4	8.54
5	8.82

a Sugar conc. is 10%.

The relation between the ethanol production rate and the brix degree of the sweet sorghum juice has been intensively studied. In Wujih area AM-4, cultivated in three planting densities, is analysed and a relation of $r=0.9952$ and a regression of $\hat{y}=1.6849x-1.9865$ are found. Here $\hat{y}=$% alcohol and x =brix degree. In Taichung area ther elationship of six different sweet sorghum varieties including AM-4 is as follows: $r=0.8585$ and $\hat{y}=0.6949x-3.1927$. Furthermore, 13 other sweet sorghum lines with four replications are also studied and a relation of $r=0.9384$ and a regression of $\hat{y}=0.66226x-2.61642$ are established. A highly significant relation exists between the brix degree of

the juice and the ethanol production rate. With the brix degree known the ethanol production rate of sweet sorghum can be estimated. Increasing one brix degree can produce 0.66% more absolute ethanol (Fig. 2).

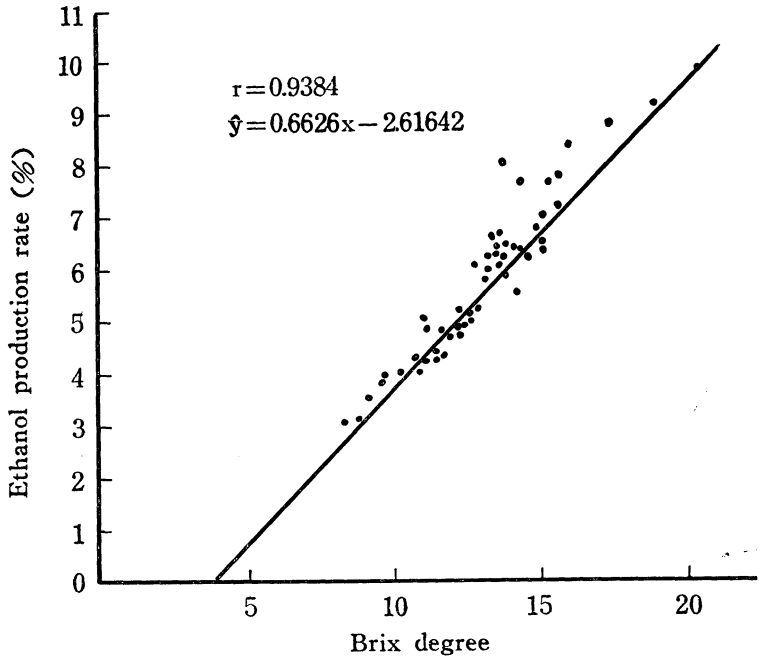

Fig. 2. Relationship between ethanol production rate and brix in sweet sorghum juice

To evaluate the output-input coefficient from the alcohol production of sweet sorghum, several references are taken into account. The agricultural costs of sweet sorghum AM-4 is the same as that of grain sorghum [5,6]. The juice production cost is calculated on the basis of using small scale pressing machines. The fermentation and distillation costs are based on the information source of Lipinsky *et al.* [cf,13]. The liter absolute alcohol costs NT $400. The seed cost per kg is NT$14. The bagasse costs NT$1,000 per ton. According to these informations the total input is NT$301,818/ha and the total outpt is NT$435,000/ha. The main input item is the cost of fermentation, being 59.3% of the total input costs. And the main output item is the ethanol production (84.4%) (Table 10).

Table 10. Estimated total input and output for alcohol production of sweet sorghum AM-4

Item	NT$/ha	%
Input		
Agricultural costs	57,313	18.99
Juice production process	65,527	21.71
Fermentation & Distillation	178,978	59.30
Total input	301,818	100.00
Output		
Ethanol production	367,000	84.37
Seed production	28,000	6.44
Bagasse production	40,000	9.19
Total output	435,000	100.00

The output-input coefficient is 1.44 for sweet sorghum and 1.15 for grain sorghum [6]. That means NT$1.44 can be gained if NT$1.00 is invested for the alcohol production from sweet sorghum. The hectare profit of alcohol production from sweet sorghum is nearly 16 times as high as that from grain sorghum (Table 11).

Table 11. The output/input coefficient of sweet sorghum and grain sorghum

Sorghum Type	Output (NT$/ha)	Input (NT$/ha)	coefficient
Sweet sorghum	435,000	301,818	1.44
Grain sorghum	65,814	57,313	1.15

Conclusion

The juice of AM-4 stalk contains enough sucrose and reducing sugars and can be used as a suitable solution for alcohol fermentation with *S. cerevisiae*. Besides *S. cerevisiae* yeast strain Sf can also produce more than 9% ethanol rate within two days. This means that more than 90 ml absolute alcohol can be obtained from one liter of juice. The needed temperature for *S. cerevisiae* is 30°C, which can be easily achieved by using the abundant solar energy in Taiwan. Using good sweet sorghum lines such as AM-4 and good yeast strains, it is possible to develop an alcohol industry here in the near future.

The alcohol yield per hectare of AM-4 juice found in this study amounts only 918.8 liters, while the result of the American Battele Institute is 3,554 l [2]. The possible difference between these two results is the quantity of the produced juice, which runs up to only 18-20% of stalk biomass by small scale pressing machines used in this study, but it can be as high as 60-80% if large scale juice production methods taken in sugarcane factories are used. If these methods would be used for sweet sorghum in Taiwan, the alcohol yield per hectare could excess the niveau of the U. S. A. Furthermore, the costs for fermentation and distillation can be decreased through the improvement of new yeast strains and new fermentation techniques such as yeast cell immobilization and the automatic control, etc.

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甜高粱生質產量
莖汁品質與酒精生成潛力之研究[1]

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摘 要

能源作物能在短時間內與常溫常壓下生生不息地吸收儲存太陽能量，是各國在尋求替代能源時的重要研究發展方向之一。本研究將就一優良甜高粱品系AM-4之生質生產量，莖汁理化性質及其酒精生成潛力來評估它在本省成爲優良能源作物的可能性。

由試驗結果顯示，AM-4具有下列特點：豐富的生質產量（44.4—46.1t/ha）甚高的錘度（18.2—23.2）及蔗糖量（11.0—12.0%），生育日數不長（4個月），廣大的適應能力，以及其汁液能被直接利用來生產酒精，且由其莖汁酒精醱酵結果得知，至少有兩種酵母菌株(*S. cerevisiae* 及 *S. f.*）能利用 AM-4 汁液在兩天之內生產9%以上的高酒精率，而所須溫度只 30°C，將由於利用本省無時不有的太陽能而大幅降低其酒精生產費用。由上觀之，AM-4 在本省將成爲一優良的酒精生產原料來源。

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